97. Resolving central metabolism of wild type and engineered *Yarrowia lipolytica* by ^{13}C- metablic flux analysis with multiple isotopic tracers

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Project Goals: The project aims to develop ^{13}C-metabolic flux analysis (^{13}C-MFA) methodology for flux determination in *Yarrowia lipolytica* with high resolution and accuracy. The generated flux information will be applied to identify gene targets and develop metabolically engineered cells for biodiesel production.

The oleaginous yeast *Yarrowia lipolytica* can accumulate large quantities of lipids in the form of triacylglycerol (TAG) in its lipid body, making it an attractive host for biodiesel production. To increase TAG production, *Y. lipolytica* needs to be engineered through genetic manipulations, e.g. gene knockout and over-expression. Thus, the determination of *in vivo* metabolic fluxes can provide critical information for the identification of target genes and bottle- neck pathways and the evaluation of engineered strains.

Here, we constructed a metabolic model of *Y. lipolytica* and performed ^{13}C-MFA, which can estimate *in vivo* metabolic fluxes, including net fluxes as well as the rates of reverse reactions in central metabolism. Generally, ^{13}C-MFA utilizes substrates with ^{13}C-atoms, i.e. isotopic tracers which are incorporated into intracellular metabolites by biochemical reactions and the labeled metabolites are analyzed with mass spectrometry. However, one type of isotopic tracer provides only limited flux observability in terms of continuous atom transitions in central metabolism. To overcome this shortcoming, we combined analysis of multiple labeling data sets obtained by parallel experiments with different ^{13}C-labeled tracers. As a result, we achieved high flux observability in *Y. lipolytica* central metabolism with high resolution and accuracy compared to individual tracer experiments.

Using the combined analysis with ^{13}C-MFA, we evaluated wild type and engineered *Y. lipolytica* over-expressing acetyl-CoA carboxylase (ACC) and diacylglycerol acyltransferase (DGA). We successfully estimated most of the fluxes through the pentose phosphate pathway (PPP), glycolysis, citric acid cycle and lipid metabolism, including reaction reversibility, with the combined analysis. Furthermore, the flux maps indicated the metabolic rewiring of engineered cells for production of lipids. In the case of NADPH generating pathways, we observed a dramatic increase in the oxidative PPP flux in the engineered strain relative to the wild type strain. In contrast, we observed little change in the malic enzyme flux in the ACC DGA strain. Taken together, these results suggest the oxidative PPP is the primary source of NADPH for lipid over-production. In sum, the high resolution and accurate flux determination by combined analysis allowed us to evaluate engineered cells and to identify key enzyme targets for further engineering

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